Agronomic and phenotypic assessment of the Late Blight Resistant Potato Event Vic.172 grown in three locations in Uganda during 2020.

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Abbreviations

CFT	Confined Field Trial
DAP	Days After Planting
DNA	Deoxyribonucleic Acid
LB	Late Blight
ML-CFT	Multilocational CFT
PDA	Personal Digital Assistant
RB	R protein of the *RB* gene conferring late blight resistance
RBLB2	R protein of the *Rpi-blb2* gene conferring late blight resistance
RS	Regulatory Study
RVNT1	R protein of the *Rpi-vnt1.1* gene conferring late blight resistance
T-DNA	Transfer DNA
ZARDI	Zonal Agricultural Research and Development Institute

Abstract

The late blight resistant potato Event Vic.172 was developed via *Agrobacterium*-mediated transformation of the popular Ugandan variety Victoria with the insertion of a T-DNA containing 3 *R* genes from wild relatives of the potato and the *nptII* selectable marker gene. The intended effect is to confer resistance to the late blight disease that is responsible for 13-60% of yield loss in Uganda. Here, we assessed whether the genetic engineering has led to any significant unintended effects on the phenotype and agronomic performance of Event Vic.172 by comparative analyses with the potato variety Victoria from which the transgenic event was developed. Regulatory trials were conducted at three locations in Uganda. The trials were set with identical tuber seed quality and growth conditions for both genotypes, Event Vic.172 and Victoria, which included fungicide sprays during the Late Blight disease season. From sprouting tuber seeds to harvest of tubers, phenotypic criteria were evaluated mostly weekly at the plot level. During the maturity period and at harvest, parameters to assess agronomic performance were collected weekly. The data from three plot replications per genotype and from three locations were analyzed using mixed model statistical analyses. During the 1st season, the multilocational confined field trial revealed few statistically significant differences between Event Vic.172 and Victoria. The number of flowers per plant which appeared to be less abundant for Event Vic.172 at each location and the number of interjected leaves which were slightly more abundant in the Event Vic.172. Other observed minor differences on color and vigor are likely to be evaluator bias. These phenotypic differences could be due to the clonal propagation of the crop and change over time. The total number of tubers per plants was slightly lower for the event Vic.172 but was mainly due to non-marketable tubers. The difference in marketable yield between Vic.172 and Victoria were a slight yield increase of Vic.172 at Kachwekano ZARDI (6.3%) which is the main potato growing area of Uganda whereas at the other two locations, Rwebitaba and Buginyanya, Event Vic.172 had lower yield by 12.3% and 14.6% respectively. The average yield reduction of Event Vic.172 across all three locations is only 2.7%. This small difference and the lack of the same trend across locations suggest it is not due to genetic difference between Vic.172 and Victoria. Overall, the 1st season of multilocational confined field trials (ML-CFT-3) revealed small differences between the Event Vic.172 and Victoria which did not significantly affect the phenotype and agronomic performance of the event Vic.172.

Objectives of the study

Potential impact of the introduction of the three *R* genes into the genome of potato Event Vic.172 on agronomic and phenotypic characteristics, with the exception of the introduced late blight resistance trait, was assessed in field conditions in three locations in Uganda during 2020.

Specifically, the objectives of this study are to:

- Compare growth, morphology, architectural traits between Vic.172 and Victoria grown under identical field conditions.
- Compare reproductive ability from flower and volunteer between Vic.172 and Victoria grown under identical field conditions.
- Compare the tuber yield between Vic.172 and Victoria grown under identical field conditions.

Introduction

Potato (*Solanum tuberosum* L.) is one of the important food and cash crop which is grown by small-scale farmers in the highland regions of Uganda. Based on 2018 survey, the total production of Irish potatoes was estimated to be 327,300 MT (metric tons), from an estimated cultivated area of 111,100 hectares (Ha) of which nearly 60% produced from south western highlands (UBOS 2020). Potato production has been continuously on the rise 155,000 MT in 2005/06 to 327,300 MT in 2018. The on-station potato productivity in Uganda is estimated at 25-30 t/ha, however, the national average is 3.5 t/ha (UBOS 2020). The main reason for low yields is because of late blight disease that is responsible for 13-60% of the yield loss and without chemical control, late blight disease can even lead to complete yield loss (Kakuhenzire 2009; Namugga 2017).

In response to the production challenges due to late blight disease, CIP developed transgenic events from potato varieties of which a dozen were evaluated by NARO both in the screenhouse and in the field and proved to have complete resistance to late blight (Ghislain et al., 2019). The potato event Vic.172 was developed at the advanced biotechnology laboratory (CIP-ABL) in Peru in 2015 by methods of biotechnology to express three naturally-occurring resistance genes (*R* genes) that confer protection against the late blight disease. Potato Late Blight disease is caused by the oomycete, *Phytophthora infestans*, a serious pest of potatoes, and results in significant yield loss as well as high costs of preventative fungicide applications. Cultivation of the event Vic.172 resistant to the late blight disease will increase income for farmers through the reduction of production losses and costs associated with the use of fungicides, and potentially decrease of the human and environmental impact of use of chemical fungicides.

Event Vic.172 derives from the CIP breeding clone (CIP381381.20) which was released in 1991 and became the popular Ugandan potato variety 'Victoria'. It contains three resistance genes (3*R*) from wild relatives of the potato and a selectable marker gene as a single T-DNA inserted into the genome. Victoria potato line was transformed using common bacteria Agrobacterium tumefaciens with the plant transformation plasmid vector pCIP99. The T-DNA of this vector contains three resistance (*R*) genes previously isolated from wild species (*RB* and *Rpi-blb2* from *Solanum bulbocastanum* and *Rpi-vnt1.1* from *S. venturii*) that provide resistance to late blight, and the *nptII* gene which confers resistance to kanamycin on tissue culture selective media and was used as a selectable marker during event development.

The event Vic.172, was selected for potential release based on strict selection criteria that include intact stable insertion into the genome (see RS-1, 2 and 3), trait efficacy evaluation in greenhouse and small scale trials in the field in Uganda (see RS-4). Therefore, to ensure that the transgenic Event Vic.172 has equivalent phenotype and agronomic performance to the variety Victoria it derives from, we conducted field studies in Uganda at locations representing three different agroecological zones over the seasons from October 2019 to January 2020.

Study dates

14th October 2019 to 31 January 2020

Start date = importation of 1,000 seed tubers from CIP BecA Kenya to NARO Kachwekano ZARDI Uganda of Vic.172 and Victoria (50/50).

End date = after harvest of all regulatory plots at all 3 locations in Uganda.

Materials

Variety Victoria

The Ugandan variety Victoria is the CIP breeding lines CIP381381.20 which was obtained from the CIP Genebank in Peru. Their identical identity was verified using 9 SSR markers and a sample of potato variety grown under the same name in Africa in April 2010. We extracted DNA at CIP-BecA Kenya leaves provided from the variety 'Asante' from KEPHIS Nairobi Kenya ('Asante' is the Kenyan name of the variety Victoria).

Event Vic.172

The Event Vic.172 was produced at CIP-ABL in Peru in 2015 by genetic transformation of the variety Victoria using the pCIP99 binary transformation vector and the hyper virulent strain EHA105 of *Agrobacterium tumefaciens* (Ghislain et al., 2019). It is stored in CIP Genebank as the CIP accession CIP 820046.172. The plant transformation vector pCIP99 was assembled at CIP-ABL Peru in 2010 with a T-DNA composed of the *RB* and *Rpi-blb2* genes from *Solanum bulbocastanum* and *Rpi-vnt1.1* from *S. venturii* and the *nptII* selectable marker gene in pCAMBIA1300 (GenBank #: MN164628; see also RS-1).

Seed tubers

Potato tubers of the Event Vic.172 and Victoria were produced from potted tubers in the screenhouse located within the confined field at Kachwekano ZARDI. After harvest, tubers were left in the dark for 2 months until sprouts of 10-13 mm were observed. Tubers with sprouts of uniform sizes were chosen for planting the regulatory plots. Tuber seeds were transported by road to Rwebitaba ZARDI 1 day after planting in Kachwekano ZARDI and to Buginyanya ZARDI 2 days after planting in Rwebitaba ZARDI.

Locations

Three locations for multi-locational confined field trials (ML-CFT) were chosen in consultation between NARO potato experts and the National Biosafety Committee of Uganda. Inspections were made prior to and after construction of the confined field, screenhouse, store and office in accordance with the requisite of the NBC guidelines (UNCST 2011). Their geographic location and description of the three locations are presented below (Figure 1, Table 1).

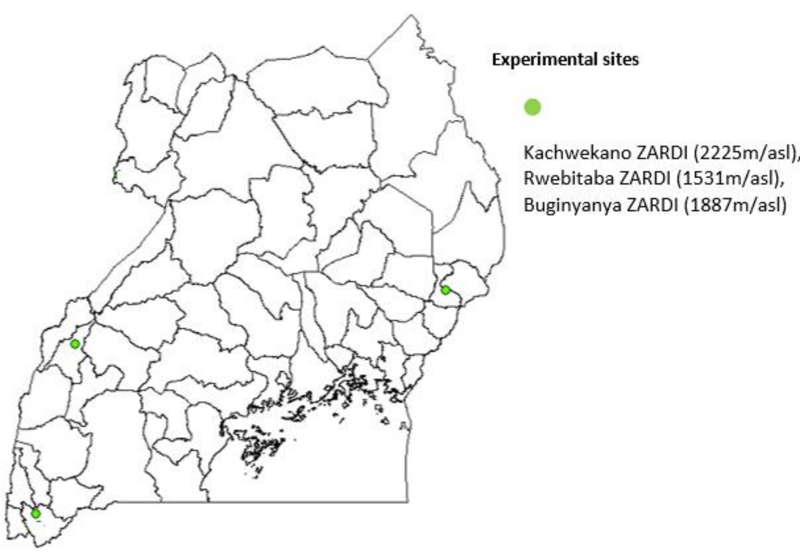


Figure 1: Map of Uganda indicating the position of the 3 locations of the confined field trials used in the study.

Kachwekano ZARDI- KaZARDI (near Kabale): located in Kabale district, Bubare sub-county, Kagarama village, Lake Bunyonyi Road, at an altitude 2,225 masl. It belongs to the south western highland agro-ecological zone (SWHAEZ). It is cool and windy and received 1,126.6 mm of rain for the year 2019. The rainfall pattern was bimodal with rain being more distributed during the second season than the first season. The mean annual minimum temperature for the year 2019 was 12.7°C and the mean maximum temperature was 24.4°C. The soils are fertile and sandy loam.

Rwebitaba ZARDI (near Kabalore): located in Kabalore district near the city of Fort-Portal at an altitude 1,531 masl. The area covers three agricultural production zones where potatoes are grown in the highland areas. The climate is tropical within which exists a wide variation of climatic conditions including wet lowland, dry savanna, wet mountain forest and alpine forest. The area experiences a bimodal rainfall pattern with average annual rainfall ranging from 750-1,000 mm. The area received about 1,680.2 mm of rain for the year 2019 and minimum temperature of 15.3°C, and maximum temperature of 26.1°C. The site has loamy soils with coarse texture.

Buginyanya ZARDI – BugiZARDI (near Bulambuli): located in Bulamburi district on the slopes of Mt. Elgon at an altitude 1,887 masl. It belongs to the south eastern highland agro-ecological zone (SEAEZ). The rainfall is also bi-modal and for the year 2019, the area received a total of 2,557.7 mm and mean monthly minimum temperature of 12.7°C and maximum temperature of 24.5°C. The site has rich soils and is suitable for potato growing. The site soils were dominated by ferrisols.

Table 1: Location coordinates and planting/harvest dates for the Season 1 of regulatory trials

Location	GPS Coordinates	Planting Date	Harvest Date
Kachwekano ZARDI	Latitude 1°15'15.98"S Longitude 29°56'33.35"E	14 October 2019	27 January 2020
Rwebitaba ZARDI	Latitude 0°41'36.81"N Longitude 30°19'57.76"E.	15 October 2019	29 January 2020
Buginyanya ZARDI	Latitude 1°16'50.41"N Longitude 34°22'19.25"E	17 October 2019	31 January 2020

Methods

Field trials

At all trial sites, a randomized complete block design (RCBD) was adopted using three replications. This multilocational confined field trial is the 1st season of regulatory trials for Event Vic.172 and is referred to as ML-CFT-3.

Each plot of the trial, separated from each other by 1 m, consisted of five rows at spacing of 75 cm. 10 seed tubers were planted at spacing of 30 cm within rows. Plots of 3 m X 3 m were made within the CFT field at Kachwekano ZARDI, Rwebitaba ZARDI and Buginyanya ZARDI using the same layout (Figure 2). The field for the regulatory trials was distant by 2 m from other plots.

Plots were planted by digging straight trenches of about 10 cm deep. Fertilizer was applied manually at a rate of 100 kg/ha (NPK 17:17:17) and then sprouted tubers were placed into the trenches with sprouts facing upwards and then covered making a mound on the tubers. All plots were labeled according to the field layout using barcoded water-proof labels.

Standard crop management practices were followed. Weeding was done 4-5 times while control for cutworms, aphids and leaf-miners was done 2-3 times using systemic insecticide Rocket (profenofos 40% + cypermethrin 4%). All plots of the regulatory trials were sprayed during the LB disease period with Ridomil gold (Metalaxyl-M 40 g/kg Mancozeb 640 g/kg) in alternation with Mancozeb (Mancozeb 80% w/w) on weekly basis depending on weather conditions at each site. The start of spraying and its frequency was based on the appearance of the typical LB-lesions on leaves of Victoria potato plants. The plots flanking those of the regulatory trials were not sprayed with fungicides.

Crop management of regulatory trials

Tuber seeds were produced in the screenhouse within the confined field trial at Kachwekano ZARDI from tubers shipped by CIP-BecA in Kenya in early 2019. These were produced from the stock of Vic.172 and Victoria stored *in vitro*. Tuber seeds for the multilocational CFT were selected to be of similar size and sprout vigor.

Planting was made on the 14th October 2020 at Kachwekano ZARDI, 15th October 2019 at Rwebitaba ZARDI, and 17th October 2019 at Buginyanya ZARDI. Fertilizers were applied only at planting whereas weeding, insecticides, fungicides were applied as needed during the crop season (Annex 1). Weather data were obtained for the year 2019 until harvest (Annex 1). Each regulatory trial was flanked by plots of Victoria and Vic.172 without fungicide treatments to demonstrate trait efficacy.

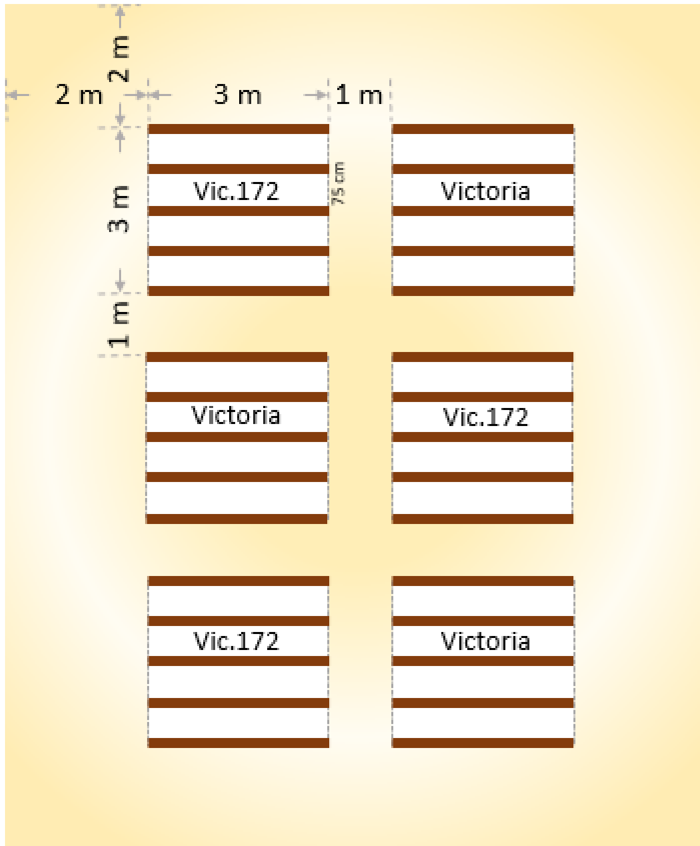


Figure 2: Regulatory trials layout for all three locations in the 1st season 2019-2020 (ML-CFT-3).

Phenotype and Agronomic performances

The Event Vic.172 and the variety Victoria were characterized for their phenotype using standard CIP charts and scales for growth habit, leaf shapes, stems pigmentations, flower colors and tuber shapes, fresh tuber color, the eye depth and eye color. Their agronomic performances were assessed during the potato life cycle which starts with planting of sprouted tubers and ends with the harvest (Figure 3).

Data was collected during the four phases: plant emergence, plant and foliage growth, flowers and berries formation, and bulking of tubers. The period from planting of sprouted tubers to the end of bulking is referred to maturity which is an intrinsic characteristic of the variety. We used NARO standards operating protocols for assessing agronomic performances. Data were recorded weekly using handheld personal digital assistant (PDA) (Motorola 9500 Series MC9596) and archived in the central point at Kachwekano ZARDI and later transferred to CIP for data verification.

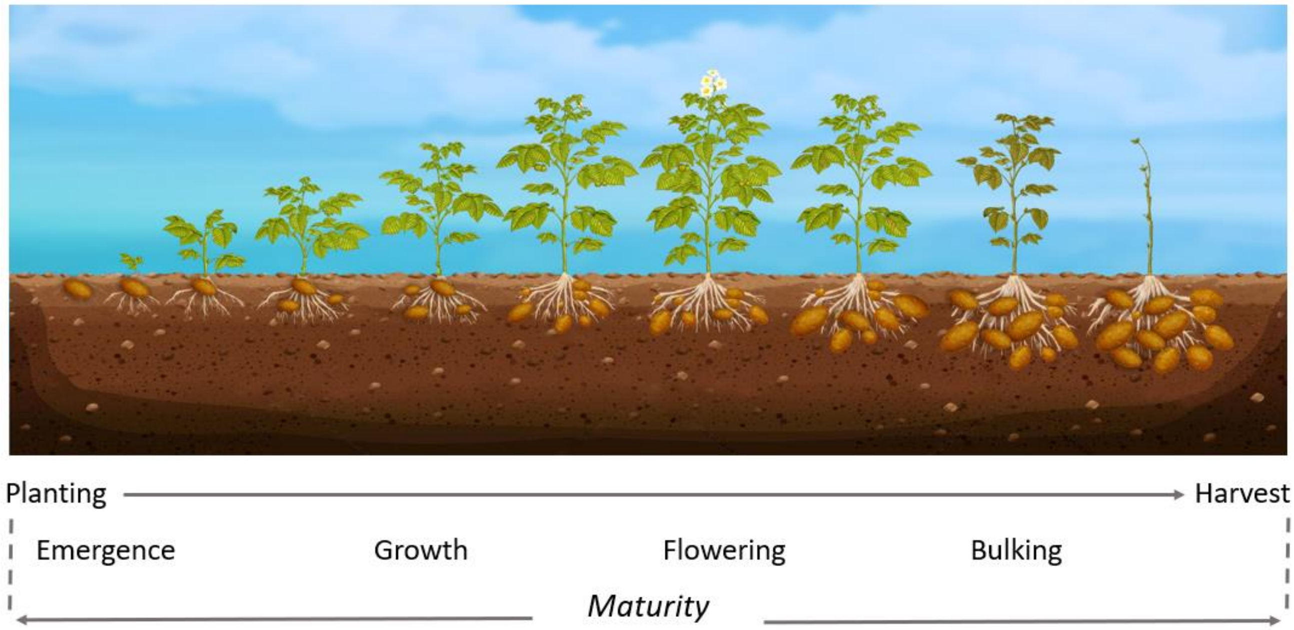


Figure 3: Life cycle of the potato starting with planting of tubers with sprouts ending with harvest after dehaulming the potato crop.

Sprouting

The seed tubers were kept in aerated plastic trays under diffuse light at Kachwekano ZARDI where seeds are produced for all three locations. The seed tubers for Vic.172 and Victoria were able to give 3-4 sprouts within two to three months after harvesting. The right stage of planting was achieved when the tuber sprouts were 1-1.2 cm long.

Emergence

From the day of planting, observations of emergence of sprouts above the soil were made weekly for each plot until plant development was well established around 50 DAP.

Phenotype

All observations were done weekly at the plot level by estimating the average value of all plant and organ observed. Stems were evaluated for their color (1 = light green, 2= green, 3 = dark green, 4 = purplish green), wing (0 = absent; 1= present) and were counted per plant 50 days after planting (DAP). Leaves were evaluated by their average number of leaflet per leaf, texture (1= smooth, 2= smooth hairy 3= rough, 4 = rough hairy), Interjected leaf numbers, color (1 = light green, 2= green, 3 = dark green), and openness (1 = open, 2 = closed). Plant vigor was assessed 45 DAP (1 to 5 scale: 1=poor vigor, 3=average vigor,5=excellent vigor). Flowers were observed by estimating their average number, and their color evaluated (1 = white, 2= light purple, 3= purple). Finally, the number of berries was estimated at 90 DAP. At harvest, tubers were characterized by observing medium and large tubers for their shape, skin and flesh color.

Plant and foliage growth

Potato growth was estimated by assessing the average plant height and width for each of the 5 rows and then averaged. Data were collected at plot level.

Yield

After bulking, the aboveground parts of the plants are cut off (dehaulming) and incinerated. Two weeks after, the tubers were dug out using grub hoes and kept all together for each plot. Tubers were classified as follows: category 1 are small tubers less than 3 cm, category 2 are medium tubers between 3-4.5 cm, and category 3 are large tubers above 4.5 cm. Number of tubers of each category was counted and weighed. The number and weight of rotten tubers and tubers with defects were also counted at plot level. Yield assessment was conducted using the data for all categories as total yield and category 2 and 3 as marketable yield. Total and marketable tuber yield in kilograms per plot was standardized to a hectare basis after correcting for missing hills by dividing the weight of harvested tubers by 6.75 and multiplying the quotient by 10 to obtain yield in tons per hectare (t/ha).

Statistical methods

Regulatory plots of the Event Vic.172 and the variety Victoria were subject to the same single treatment with a randomized complete block design at each of the 3 locations. Data used for the present study are provided separately (RS-8 Data for Tables and Figures).

Statistical analyzes were performed using a mixed model approach, with the effects of locations and genotypes (Vic.172 and Victoria) being considered as fixed, and the effect of blocks within locations as being random. The variables that were evaluated in different months and/or weeks, were treated as repeated values over time, and this effect (correlation between values in the same experimental plot at different times) was modeled in the residual matrix. The prediction of fixed effects was made by obtaining BLUE's (Best Linear Unbiased Estimator).

Two models of variance were used, a uniform model, which is equivalent to the model of split plot design, which assumes that the correlations between repeated values are constant over time, and a first-order autoregressive model, which these correlations are assumed to decrease over time. To compare the models, Bayesian Information Criteria (BIC) and REML log-likelihood were calculated. The significance tests for fixed effects were performed using the Wald statistic test. The analyzes were performed using the ASReml-R version 4.1.0 software. The output file is provided as supplementary data as a separate file (RS-8 Supplementary data).

Results

The phenotype and agronomic performance of Event Vic.172 and the variety 'Victoria' were examined for sprouting, emergence, 14 phenotypic criteria, plant and foliage growth, number of total and marketable tubers per plants, and yield.

Sprouting

The seed tubers had sprouted after 2 months under room temperature and diffuse light at Kachwekano ZARDI without any physiological stress or chemicals to interrupt dormancy. There was no significant difference between Vic.172 and Victoria in number of sprouts per tuber and in the sprout length by the end of 3 months (Figure 4).

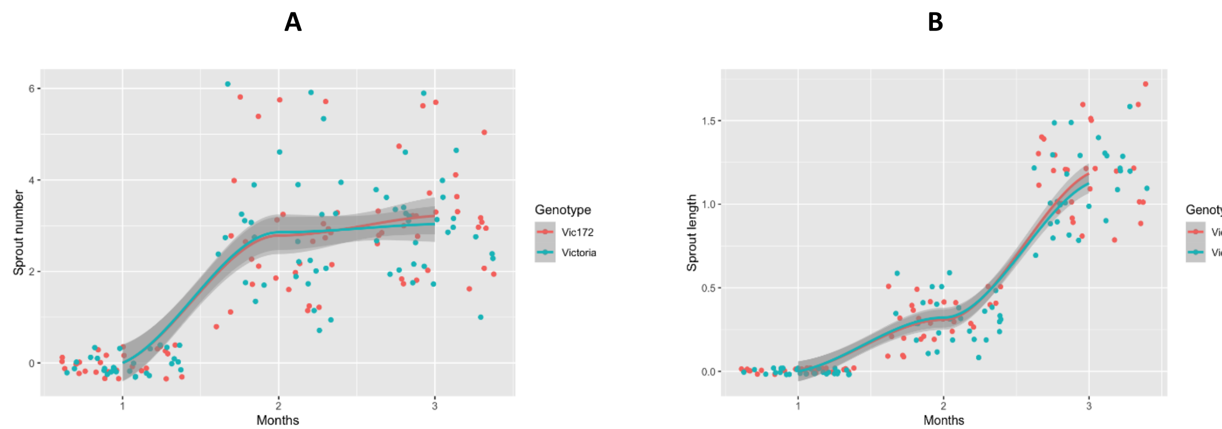


Figure 4: Sprouting efficiency of the Event Vic.172 and Victoria measured by sprout number (A) and sprout length (B) over a 3-month period in 2020 (17 March – 17 June 2020).

The statistical analysis was conducted on sprout number and sprout length (Table 2). The best fit model for the variable sprout number was the *ar1* model, and for sprout length it was the *un* model (supplementary data). For both variables, no significant differences in sprouting characteristics were observed between the Event Vic.172 and the variety Victoria.

Table 2: Sum of squares, mean squares, degrees of freedom, coefficient of experimental variation and BLUE's of sprout number and sprout length between Event Vic.172 and Victoria evaluated in Kachwekano ZARDI in 2020.

Source of variation	DF(1)	SS(2)	
		Sprout number	Sprout length
Genotypes	1	0.0747^{ns}	0.0100^{ns}
Month	2	485.7176**	43.9165**
Genotype by Month	2	0.8310^{ns}	0.0506^{ns}
Residual (MS)(3)	162	1.0321	0.0253
Mean		1.98	0.49
CV4		52.17	5.16

BLUE for Vic.172	2.00	0.50
BLUE for Victoria	1.96	0.48

(1) DF = Degrees of freedom, (2) Sum of squares, (3) Mean squares, (4) coefficient of variation, ns not significant by the Wald test; **significant at 1 % probability by the Wald test.

Emergence

All the material started to emerge 2 weeks after planting, 50% emergence was reached at 3 weeks after planting, and its maximum at 6 weeks after planting (Table 3).

Table 3: Emergence of Vic.172 and Victoria in regulatory plots in the multilocational trials.

Location[1]	Genotype	% emergence by weeks after planting										
		1	2	3	4	5	6	7	8	9	10	11
KaZARDI	**Vic.172**	-	1.3	37.3	86.7	96.0	98.0	98.0	94.7	98.7	98.7	98.7
	Victoria	-	0.7	52.7	81.3	92.0	92.7	94.7	98.7	94.0	94.0	94.0
Rwebitaba ZARDI	**Vic.172**	-	0.7	50.7	94.7	98.7	98.7	99.3	99.3	99.3	99.3	99.3
	Victoria	-	8.0	78.7	97.3	98.7	98.7	98.7	98.7	98.7	98.7	98.7
BugiZARDI	**Vic.172**	-	20.0	33.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Victoria	-	21.3	46.7	90.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0

(1) Locations are described in the Materials and Methods section.

A statistical analysis was conducted on the % emergence in order to test whether the emergence between the Event Vic.172 and the variety Victoria across the different locations was the same (Table 4). The best fit model was the *ar1* model (supplementary data).

The results revealed that there was no significant difference in emergence between the Event Vic.172 and Victoria at the 3 locations indicating that Event Vic.172 and Victoria had the same percentage of emergence. It was possible to detect a highly significant interaction between Genotype by Weeks on the third week only. Indeed, on third week Event Vic.172 had a lower % of emergence at all three locations. However, by the 8th week, there were no difference in emergence and all the tubers had reached 99-100 % germination.

Table 4: Sum of squares, mean squares for the percentage of emergence 8 weeks after planting evaluated in three locations (Kachwekano ZARDI, Rwebitaba ZARDI, and Buginyanya ZARDI) and two genotypes (Vic. 172 and Victoria), Uganda – 2020.

Source of variation	DF[1]	SS[2]

Location	2	7.3565*
Genotype	1	0.2742^{ns}
Genotype by Location	2	8.9742*
Week	11	10537.040**
Genotype by Week	11	182.9960***
Location by Week	22	384.4546***
Genotype by Location by Week	22	22.9638^{ns}
Residual (MS)(3)	144	1.0000

(1)DF = Degrees of freedom, (2) Sum of squares, ^{ns} not significant by the Wald test; ***, **, *significant at 0.1, 1 and 5% probability by the Wald test; (3) Mean squares.

Phenotype

After emergence, the main phenotypic characteristics of potato were scored weekly until dehaulming at week 13. The criteria chosen are those used in potato when conducting phenotypic characterization of conventionally bred potato lines for distinctness. These characteristics include stem color and wing, number of leaflets, texture of leaves, number of interjected leaves, leaf color and openness, plant vigor, and number of flowers and berries.

The data are summarized in the narrative below and summarized in Table 5. The report focusses on differences between Event Vic.172 and Victoria. The differences between weeks and locations are not reported because these are expected and have no impact on the performance of Event Vic.172 vis a vis Victoria.

Stem color was recorded as '4-Purplish Green' at Kachwekano ZARDI for all plots from week 6 until 13, '4-Purplish Green' from week 6 to 11 followed by '2-Green' and '1-Light Green' during senescence on week 12 and 13 at Rwebitaba ZARDI for all plots, and '4-Purplish Green' for all pots from week 6 until 13 at Buginyanya ZARDI. Hence, no stem color differences were notable between Vic.172 and Victoria at any location and between locations. Fading the purplish green towards senescence was observed at RwebiZARDI for both, test and control, groups.

Stem wing was recorded as '1-Present' during all time at all three locations. No differences in stem wing were observed between Vic.172 and Victoria when compared within any location, and between locations.

The average number of leaflets per leaf was recorded as 7 on week 6 and 9 on week 7 until 13 at Kachwekano ZARDI for all plots, between 6 and 11 with slight variation between plots and week at Rwebitaba ZARDI, and 7 to 9 from week 6 until 13 with slight variation between plots and week at Buginyanya ZARDI. No significant differences in number of leaflets between the Event Vic.172 and Victoria were detected at any location.

Texture of the leaves was recorded as '3-Rough' for all plots from week 6 until 13 at Kachwekano ZARDI, '2-Smooth Hairy' for all plots from week 6 until 11 and '4-Rough Hairy' for week 12 and 13 at Rwebitaba ZARDI, and '2-Smooth' for all plots from week 6 until 13 except for Vic.172 plots from 10 until 13 recorded as '4-Rough Hairy' at Buginyanya ZARDI. This Vic.172 change from smooth hairy to rough hairy observed at the time of maximum plant growth at Rwebitaba was not observed at the two other locations which points at a possible bias from the evaluator. Hence, there were no significant differences in the texture of the leaves between

Vic.172 and Victoria except after week 10 in one location which is likely either an evaluator bias or caused by environmental factors.

The number of interjected leaves was recorded as 10 to 16 on week 6 and grew to 24 to 28 on week 13 at Kachwekano ZARDI, 7 to 8 on week 6 increasing to 11.6 to 21.5 on week 13 at Rwebitaba ZARDI, and 16 to 30 on week 6 reducing slightly to 10 to 14 on week 13 at Buginyanya ZARDI. Statistical analyses revealed highly significant differences between genotypes with the Event Vic.172 bearing slightly more interjected leaves (1--3 more in Event Vic.172 than Victoria on average). This difference is attributed to clonal variation which occurs regularly in potato due to its clonal propagation. However, it is unlikely to confer significant differences in agronomic performance.

Leaf color was recorded as '2-Green' for all plots at all locations from week 6 until 13 except that they turned light green at senescence in Rwebitaba ZARDI (week 13) and Buginyanya ZARDI (week 12 and 13). Hence, there are no differences in the color of the leaves between Vic.172 and Victoria at any location, and between locations.

Leaf openness was recorded as '2-Closed' for all plots from week 6 until 13 at Kachwekano ZARDI and Buginyanya ZARDI, and '1-Open' for all plots from week 6 until 13 at Rwebitaba ZARDI. Hence, there are no differences in the leaf openness between Vic.172 and Victoria at any location.

Plant vigor was recorded as '5-Excellent Vigor' for all plots at all locations from week 6 until 13 except at Rwebitaba ZARDI where the plots with Vic.172 reduced to '3-Average Vigor' at senescence on week 12 and 13. The statistical analysis confirmed that this difference was significant only at one location during senescence. This observation could, therefore, be due to evaluator bias or to an environmental factor present only in that specific location.

Flowers were observed from week 7 to 12. The average number of flowers per plant ranged from 10 to 16 at Kachwekano ZARDI, 0 to 13 at Rwebitaba ZARDI, and 0 to 7 at Buginyanya ZARDI with slightly more flowers on Victoria than on the Event Vic.172 at each location (Figure 5).

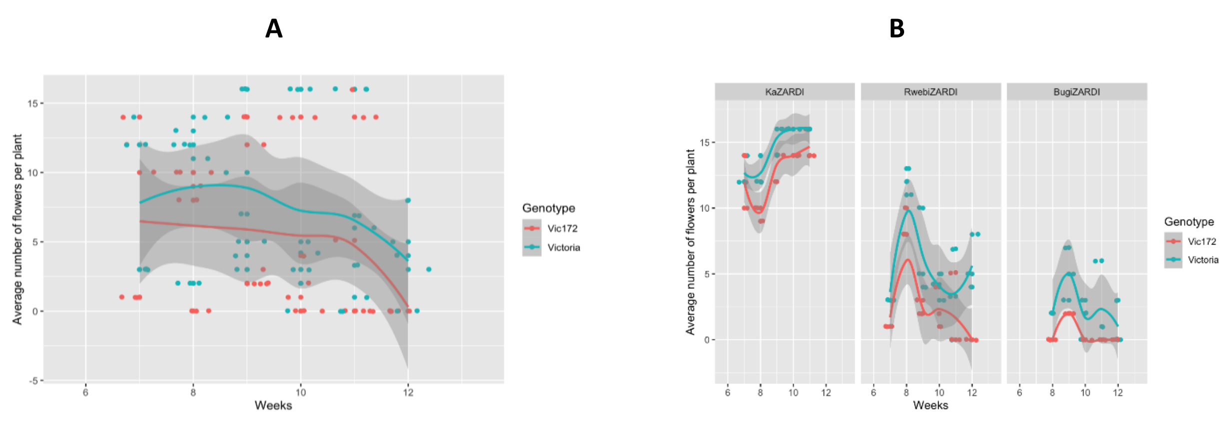


Figure 5: Degree of flowering of Vic.172 and Victoria during 1st season (ML-CFT-3) average across all 3 locations (A) and at individual locations (B) in Uganda (November 2019- January 2020).

The best model for number of flowers was *ar1* (supplementary data). There were highly significant differences in the flower formation (Table 5) across the locations, between the two genotypes, between weeks, and Genotype x Location x Weeks interaction. In all three location the Event Vic.172 produced less flowers than

Victoria. In Kachwekano, it produced 13 flowers on average per week during week-7 to week-11 when Victoria produced 15 flowers on average during the same period. In the other two locations, the numbers of flowers were much lower, but the difference were maintained 3 and 0 for Event Vic.172 and 6 and 3 for Victoria in Rwebitaba and Buginyanya respectively. Because, potato is not multiplied though seeds from flowers, this difference is not considered to have an impact on the agronomic performance.

Table 5: Mean squares for the number of flowers between genotypes and locations.

Source of variation	DF[(1)]	SS[(2)]
Location	2	1085.1476***
Genotypes	1	37.7246***
Genotype by Location	2	2.9269[ns]
Weeks	5	33.7472***
Genotype by Weeks	5	7.0710[ns]
Genotype by Location by Weeks	16	154.1019***
Residual (MS)	64	1.0000
Mean		6.28

[(1)] DF = Degrees of freedom, [(2)] Mean squares, [ns] not significant by the Wald test; ***, **, *significant at 0.1, 1 and 5% probability by the Wald test.

The color of the flower was '3-Purple' at Kachwekano ZARDI and Rwebitaba ZARDI, and 2-Light Purple' at Buginyanya ZARDI with no differences between the Event Vic.172 and Victoria. Finally, no berries were observed at any locations.

The average number of stems per plant was recorded as 2 for all plots from week 7 until 13 at Kachwekano ZARDI, 1 to 2 at week 7 increasing to 3 to 6 at week 13 with no differences associated with the genotype at Rwebitaba ZARDI, and 2 to 3 at week 6 until week 13 with no differences associated with the genotype at Buginyanya ZARDI. Hence, there are no differences in the average number of stems per plant between Vic.172 and Victoria at any location, and between locations.

Tuber shape, skin color, and flesh color did not show significant differences between Vic.172 and Victoria at any of the 3 locations. All tubers were recorded as round, red skin, and white flesh. Hence, there are no differences in the average number of stems per plant between Vic.172 and Victoria at any location, and between locations.

Table 6: Summary of the phenotype traits of Vic.172 compared to Victoria at three locations (Kachwekano ZARDI, Rwebitaba ZARDI, and Buginyanya ZARDI) from week 6 until 13 after planting.

	KaZARDI	Rwebitaba ZARDI	BugiZARDI	Observations

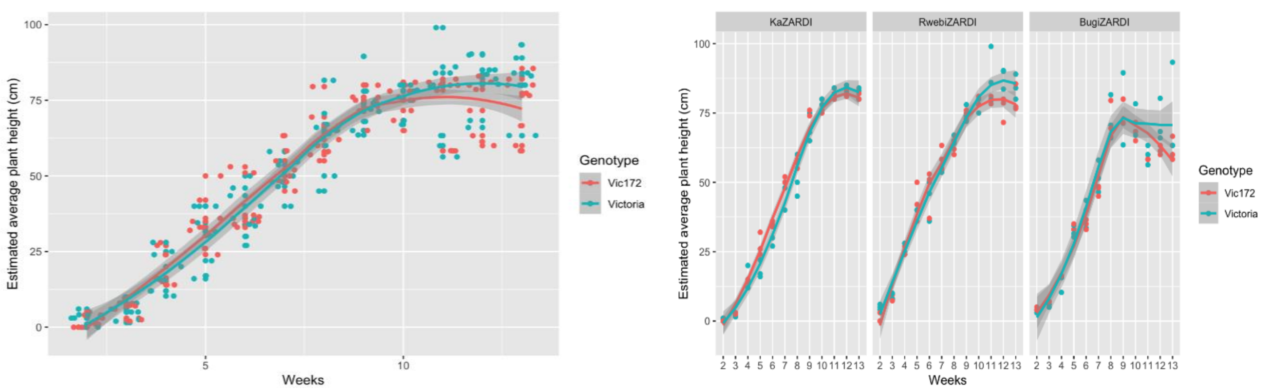
Characteristics	Vic.172	Victoria	Vic.172	Victoria	Vic.172	Victoria		Vic.172 / Victoria
Stem color	Purplish Green	Purplish Green	Purplish Green	Purplish Green	Purplish Green	Purplish Green	Minor differences between locations	No differences
Stem wing	Present	Present	Present	Present	Present	Present	No differences	No differences
Average number of leaflets per leaves	7 - 9	7 - 9	7 - 10	8 - 9	7 - 8	7 - 8	Minor differences between locations	Differences not significant
Texture of the leaves	Rough	Rough	Smooth Hairy	Smooth Hairy	Smooth Hairy	Smooth Hairy	Vic.172 getting rough hairy at senescence at one location	Differences not significant
Number of interjected leaves	14 - 25	13 - 25	7 - 20	8 - 16	13 - 20	12 - 21	On average Vic.172 had 1-3 more interjected leaf than Victoria	Differences significant
Leaf color	Green	Green	Green	Green	Green	Green	Minor differences between locations	No differences
Leaf openness	Closed	Closed	Open	Open	Closed	Closed	Minor differences between locations	No differences
Plant vigor	Excellent Vigor	Excellent Vigor	Excellent Vigor	Excellent Vigor	Excellent Vigor	Excellent Vigor	Vic.172 getting average vigor at one location	Differences not significant
Average number of flowers per plant	13	15	3	6	0	2	Less flowers on Vic.172	Differences significant
Flower color	3	3	3	3	2	2	Minor differences between locations	Differences not significant

Number of stems per plant	2	2	4	5	2	2	Minor differences between locations	Differences not significant
Tuber shape	Round	Round	Round	Round	Round	Round	No differences	No differences
Tuber skin	Red	Red	Red	Red	Red	Red	No differences	No differences
Tuber flesh	White	White	White	White	White	White	No differences	No differences

Plant and foliage growth

After emergence, the growth of the plants was observed weekly from the second week until dehaulming. Both width and height were assessed at the plot level. The progress of plant growth measured by the average of the three plots at each location shows a similar curve for Vic.172 and Victoria (Figure 6). All the potato plots obtained their maximum growth width and height around the 10th week after planting in all locations.

A



B

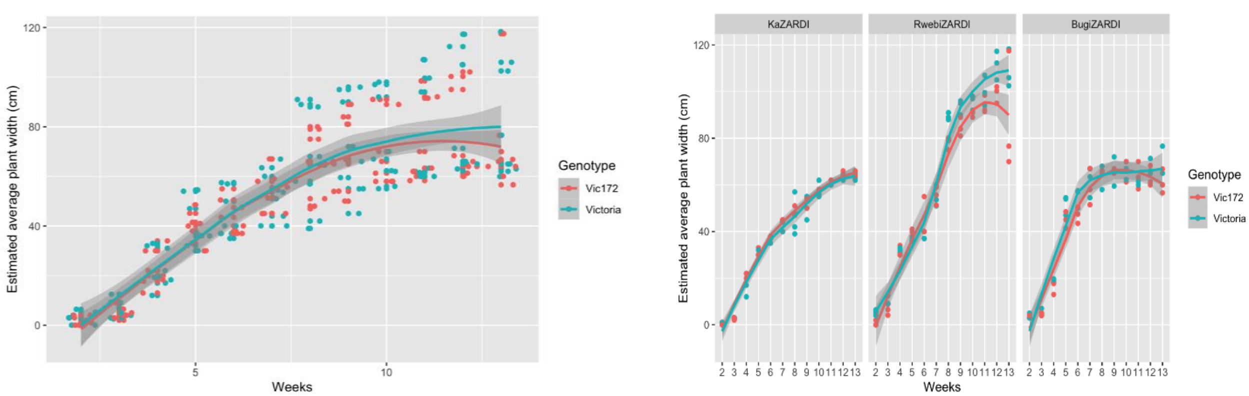


Figure 6:Growth of plant canopy cover of Vic.172 and Victoria plots at three locations (Kachwekano ZARDI, Rwebitaba ZARDI, and Buginyanya ZARDI) measured by the average height (A) and width (B) of potato rows. Plots for each location are displayed on the right.

The best fit model, both for plant growth and height, was *ar1* (supplementary data). Growth in width (canopy cover) and height was not significantly different between the Event Vic172 and Victoria. However, there was significant differences between locations with Rwebitaba having larger canopy which we attributed to environmental differences. It was also found that the triple interaction (Genotype x Location x Week) was highly significant, for both variables, indicating that the patterns of canopy coverage and height varied significantly over the weeks and between locations (Figure 6). And the differences between the genotypes were significant at the end of the cycle (week 12), for the Rwebitaba ZARDI and BugiZARDI locations but not at the Kachwekano ZARDI location.

Table 7: Sum of squares for plant growth in width and height evaluated in three locations (Kachwekano ZARDI, Rwebitaba ZARDI, and Buginyanya ZARDI) and two genotypes (Vic. 172 and Victoria), Uganda – 2020.

Source of variation	**DF**[(1)]	**Plant growth**	**Height**
Genotype	1	4.2044^{ns}	0.5483^{ns}
Location	2	318.9372***	52.4619***
Genotype by location	2	17.7970***	4.3250^{ns}
Weeks	11	6735.1092**	6926.0300**
Genotype by Weeks	11	8.9991^{ns}	31.7834***
Genotype by Location by Weeks	44	577.2283***	274.3603***
Residual	144	1.0000	1.0000
Mean		49.95	49.19
BLUE of Vic.172		48.88	48.77
BLUE of Victoria		50.02	49.61

[(1)] DF = Degrees of freedom, ^{ns} not significant by the Wald test; ***, ** significant at 0.1 and 1% probability by the Wald test.

Yield

Two weeks after dehaulming, all plots were harvested on the same day for each location: on the 27th January 2020 at Kachwekano ZARDI, 29th January 2020 at Rwebitaba ZARDI, and 31st January 2020 at Buginyanya ZARDI. The category, number and weight tubers were established per plant in each plot. Then, the same was established for each plot by adjusting to the number of emerged plants. Rotten tubers and tubers with defects were also counted and weighted. The average number of tubers per plant and yield were then calculated at each location using the average of the three replications for Vic.172, and Victoria. Total tubers (category 1, 2, and 3) and market tubers (category 2 and 3) were considered for the analyses. The yield was then estimated at hectare level (Table 7).

Table 8: Yield assessment of Vic.172 and Victoria at three locations (Kachwekano ZARDI, Rwebitaba ZARDI, and Buginyanya ZARDI) from regulatory plots of 50 potato plants with 3 repetitions. Std stands for standard deviation.

Location	Genotype	Average	Min	Max	Std
		Total tubers per plant			
KaZARDI	Vic.172	7.96	0	69	6.0988
	Victoria	8.7	2	31	4.7328
Rwebitaba ZARDI	Vic.172	6.64	2	17	3.0656
	Victoria	7.83	1	23	3.6534
BugiZARDI	Vic.172	6.27	2	14	2.4605
	Victoria	8.09	2	25	3.6249
		Market tubers per plant			
KaZARDI	Vic.172	7.04	0	67	5.7189
	Victoria	6.3	0	20	3.007
Rwebitaba ZARDI	Vic.172	5.62	1	14	2.5626
	Victoria	6.4	1	16	2.9665
BugiZARDI	Vic.172	5.53	1	14	2.2982
	Victoria	6.82	2	20	2.8404
		Total yield (t/ha)			
KaZARDI	Vic.172	38.72	0	115	19.269
	Victoria	37.36	5	105	22.1035
Rwebitaba ZARDI	Vic.172	44.59	5.99	101.43	18.947
	Victoria	50.95	4.33	104.71	21.238
BugiZARDI	Vic.172	31	7.85	76.33	14.269
	Victoria	36.91	5.43	105.76	20.5878
		Market yield (t/ha)			
KaZARDI	Vic.172	37.86	0	112	18.7252
	Victoria	35.64	0	100	21.8805
Rwebitaba ZARDI	Vic.172	43.51	4.38	101.43	18.717
	Victoria	49.69	4.33	103.99	21.0276
BugiZARDI	Vic.172	30.29	7.71	76.33	14.3127
	Victoria	35.73	5.423	101.33	20.4174

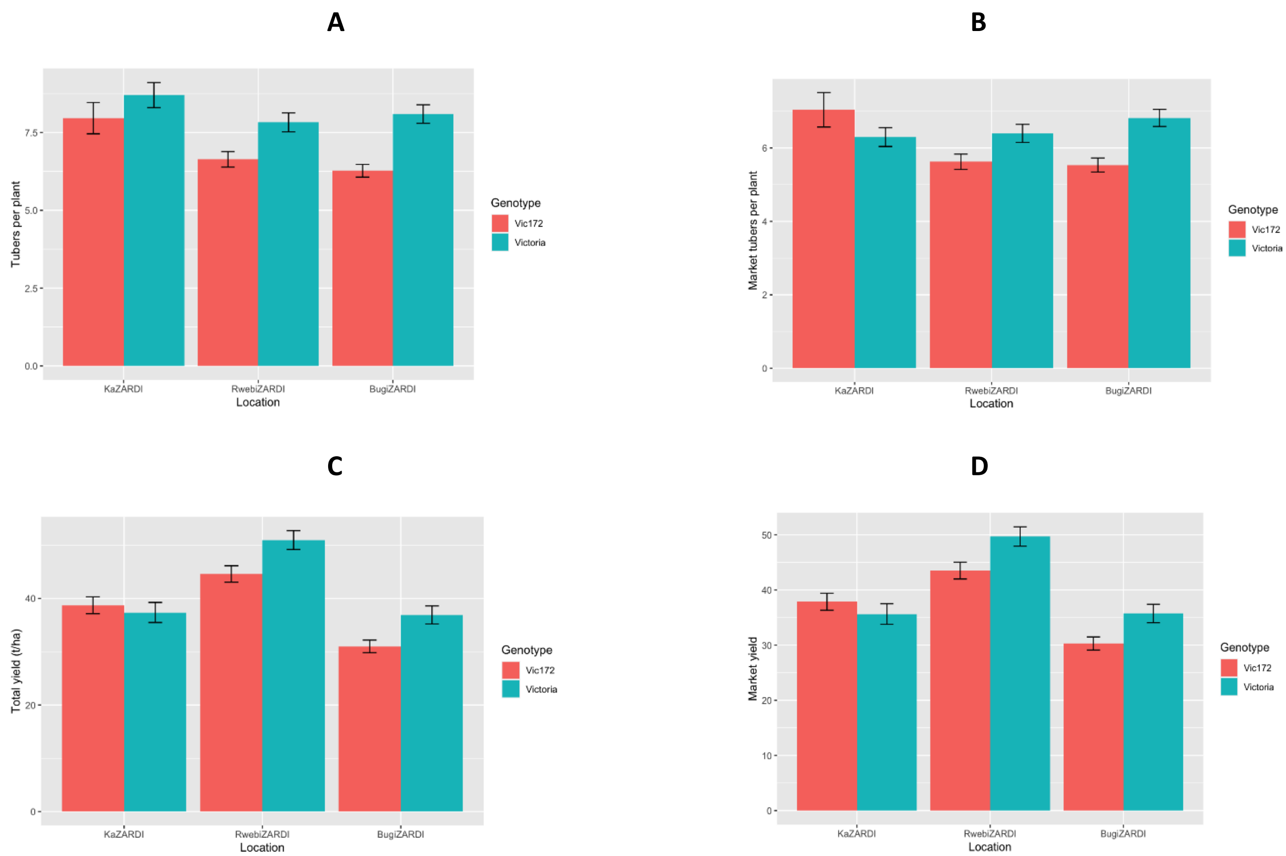


Figure 7: Yield assessment of Event Vic.172 and Victoria at three locations (Kachwekano ZARDI, Rwebitaba ZARDI, and Buginyanya ZARDI) represented here by total tuber harvested per plant (A), market tuber harvested per plant (B), total tuber yield (C), and market tuber yield (D) Market yield (A) and tubers per plant (B) for Vic.172 and Victoria estimated from plots of 50 potato plants with 3 repetitions at 3 locations.

We conducted two-way analyses on total tuber harvested per plant (TTH), market tuber harvested per plant (MKTH), total tuber yield (TTY), and market tuber yield (MKTY) (Table 9). The statistical analysis revealed that there were significant differences between genotypes, none between locations, and for genotype by locations the difference was only significant for MKTH.

The TTH for Vic.172 was 6.96 while for Victoria 8.20, a difference significant at probability of 0.1%. This difference was reduced when looking at MKTH which indicated that Vic.172 had less non-marketable tubers than Victoria.

Disregarding the GXL interaction, BLUE values[1] indicate a slightly lower market yield (MKTY) for Event Vic.172 (37.27 t/ha) than for the variety Victoria (40.30 t/ha). This represents a 2.7% reduction which was significant at probability of 5%.

[1] The best linear unbiased estimator (BLUE) is a predictor of fixed effects, in addition to having the lowest mean square error among all unbiased linear predictors.

Disregarding the genotype, Rwebitaba ZARDI produced the highest yield (46.57 t/ha) followed by Kachwekano ZARDI (36.71 t/ha) and last with Buginyanya ZARDI (33.08 t/ha). The observed location performance coincides with soil fertility and texture and weather conditions.

Table 9: Mean square for total tuber harvested per plant (TTH), market tuber harvested per plant (MKTH), total tuber yield (TTY), and of market tuber yield (MKTY) evaluated in three locations (Kachwekano ZARDI, Rwebitaba ZARDI, and Buginyanya ZARDI), Uganda - 2020. Yield is expressed in tons per hectares (t/ha).

Source of variation	DF[(1)]	SS[(2)]			
		TTH	**MKTH**	**TTY**	**MKTY**
Genotype	1	32.3686***	14.7815***	9.0443**	6.9066*
Location	2	1.9588[ns]	0.9644[ns]	7.7724*	7.3937*
Genotype X Location	2	2.6872[ns]	10.8503**	6.8892*	8.1929*
Residual (MS)	870	1.0000	1.0000	1.0000	1.0000
Mean		7.57	6.28	39.95	38.82
BLUE of Vic.172		6.96	6.07	38.15	37.27
BLUE of Victoria		8.20	6.50	41.68	40.30
BLUE of KaZARDI		8.32	6.66	37.99	36.71
BLUE of Rwebitaba ZARDI		7.23	6.01	47.74	46.57
BLUE of BugiZARDI		7.19	6.19	34.02	33.08

[(1)] DF = Degrees of freedom, [(2)] Mean squares, [ns] not significant by the Wald test; ***, ** , * significant at 0.1, 1 and 5% probability by the Wald test.

The BLUE values for total tuber harvested per plant (TTH), marketable tuber harvested per plant (MKTH), total tuber yield (TTY), and marketable tuber yield (MKTY) show slightly higher values for the Event Vic.172 in KaZARDI whereas lower values were observed for the Event Vic.172 in Rwebitaba ZARDI and Buginyanya (Table 9). For the market yield, we have an increase in yield at KaZARDI of 6.3% for Event Vic.172 whereas at Rwebitaba ZARDI and BugiZARDI the Event Vic.172 has a decrease in yield of 12.3% and 14.6% respectively.

Table 10: BLUE values for total tuber harvested per plant (TTH), marketable tuber harvested per plant (MKTH), total tuber yield (TTY), and marketable tuber yield (MKTY) evaluated in three locations (Kachwekano ZARDI, Rwebitaba ZARDI, and Buginyanya ZARDI), Uganda - 2020.

Location	Genotype	TTH	MKTH	TTY	MKTY
KaZARDI	Vic.172	7.95	7.03	38.68	37.82
	Victoria	8.69	6.29	37.31	35.59
Rwebitaba ZARDI	Vic.172	6.64	5.62	44.6	43.51
	Victoria	7.82	6.4	50.88	49.63
BugiZARDI	Vic.172	6.29	5.56	31.17	30.47
	Victoria	8.09	6.82	36.86	35.68

Rotten tubers and tubers with defects were found rarely and did not have a genotype association.

Conclusions

Regulatory trials were conducted at three locations representing the most important agroecological zones for the production of potato in Uganda. The confined field trials were conducted according to regulatory criteria, distant from potato farms, within NARO compounds, and with proper surveillance. Field slope, soil texture and fertility, and climatic conditions were different at each location. This gave rise to small differences in plant growth and yield between locations. During the 1st season (ML-CFT-3), the phenotypic characteristics studied and the agronomic performance of the Event Vic.172 compared to non-transgenic Victoria variety did not vary significantly within locations with the exception of number of flowers per plant which appeared to be less abundant for Event Vic.172 at each location and the number of interjected leaves which were slightly more abundant in the Event Vic.172. These phenotypic differences could be due to the clonal propagation of the crop and change over time. Other minor differences on color and vigor are likely to be evaluator bias. None of these phenotypic changes are expected to impact on the Event Vic.172 performance and behavior. As of the number of tubers per plants and the yield, we did obverse significant differences between Event Vic.172 and Victoria. The event Vic.172 has reduced quantity of non-marketable tubers per plant However, the differences were upward in Kachwekano and downward in Rwebitaba and Buginyanya and overall, across locations, it was only 2.7%. This small difference and the lack of the same trend across locations suggest it is not due to genetic difference between Vic.172 and Victoria. As expected , we observed differences between location with Rwebitaba ZARDI being the most fertile location followed by Kachwekano and last Buginyanya. Overall, the 1st season of multilocational confined field trials (ML-CFT-3) revealed small differences between the Event Vic.172 and Victoria which did not significantly affect the phenotype and agronomic performance of the event Vic.172.

Contributions

Abel Arinaitwe Byarugaba, Kachwekano Zonal Agricultural Research and Development Institute (ZARDI), NARO, Uganda: managed the construction of all CFT and contributed to associated institutional arrangement to perform the multi-location trials, produced tuber seeds for all three locations in Uganda, coordinated planting and field management for all three locations, recorded the field observation data in the PDA and corresponding forms, collected the samples from all three locations, coordinated their exportation to BecA Kenya, and led all data analyses reported here.

Gerald Baguma, Kachwekano ZARDI (KaZARDI), NARO, Uganda: assisted Abel Arinaitwe in all operational aspects related to the activities which took place at KaZARDI and assisted the other two locations in various operational aspects.

Douglas Mutebi, Rwebitaba ZARDI, NARO, Uganda: managed the construction of the CFT at Rwebitaba ZARDI, coordinated planting and field management for Rwebitaba ZARDI, recorded the field observation data in the PDA and corresponding forms, collected the samples, and contributed to data analyses reported here.

Faith Aharita, Rwebitaba ZARDI, NARO, Uganda, assisted Douglas Mutebi in the operational aspects related to the activities which took place at Rwebitaba ZARDI in relation to data collection and site management.

Arthur Wasukira, Buginyanya ZARDI (BugiZARDI), NARO, Uganda: managed the construction of the CFT at BugiZARDI, coordinated planting and field management for BugiZARDI, recorded the field observation data in the PDA and corresponding forms, collected the samples, and contributed to data analyses reported here.

Keneth Walimbwa, Buginyanya ZARDI (BugiZARDI), NARO, Uganda, assisted Arthur Wasukira in the operational aspects related to the activities which took place at Buginyanya ZARDI in relation to data collection and site management.

Eric Magembe, BecA/ILRI hub, CIP SSA, Kenya: coordinated the production and shipment of tuber seeds from BecA facilities in Kenya to KaZARDI facilities in Uganda and participated to the CFT monitoring visits.

Immaculate Makoko, BecA/ILRI hub, CIP SSA, Kenya: produced tuber seeds from BecA facilities in Kenya for use at KaZARDI facilities in Uganda and participated to the CFT monitoring visits.

Alex Barekye, Kachwekano ZARDI (KaZARDI), NARO, Uganda: coordinated the management of the project and activities in NARO and with national competent authorities in Uganda and contributed to the writing of the report.

Marc Ghislain, BecA/ILRI hub, CIP SSA, Kenya: oversaw the study from its original design until full completion, and lead the writing of the report(s) and publication(s).

Archiving statement

"Originals or exact copies of all raw data and pertinent information, including the original protocol, any amendments, and the final report will be archived at the International Potato Institute (CIP), at the following address: "

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Annex 1

Supplementary figures of crop management and tables of weather data at all three locations

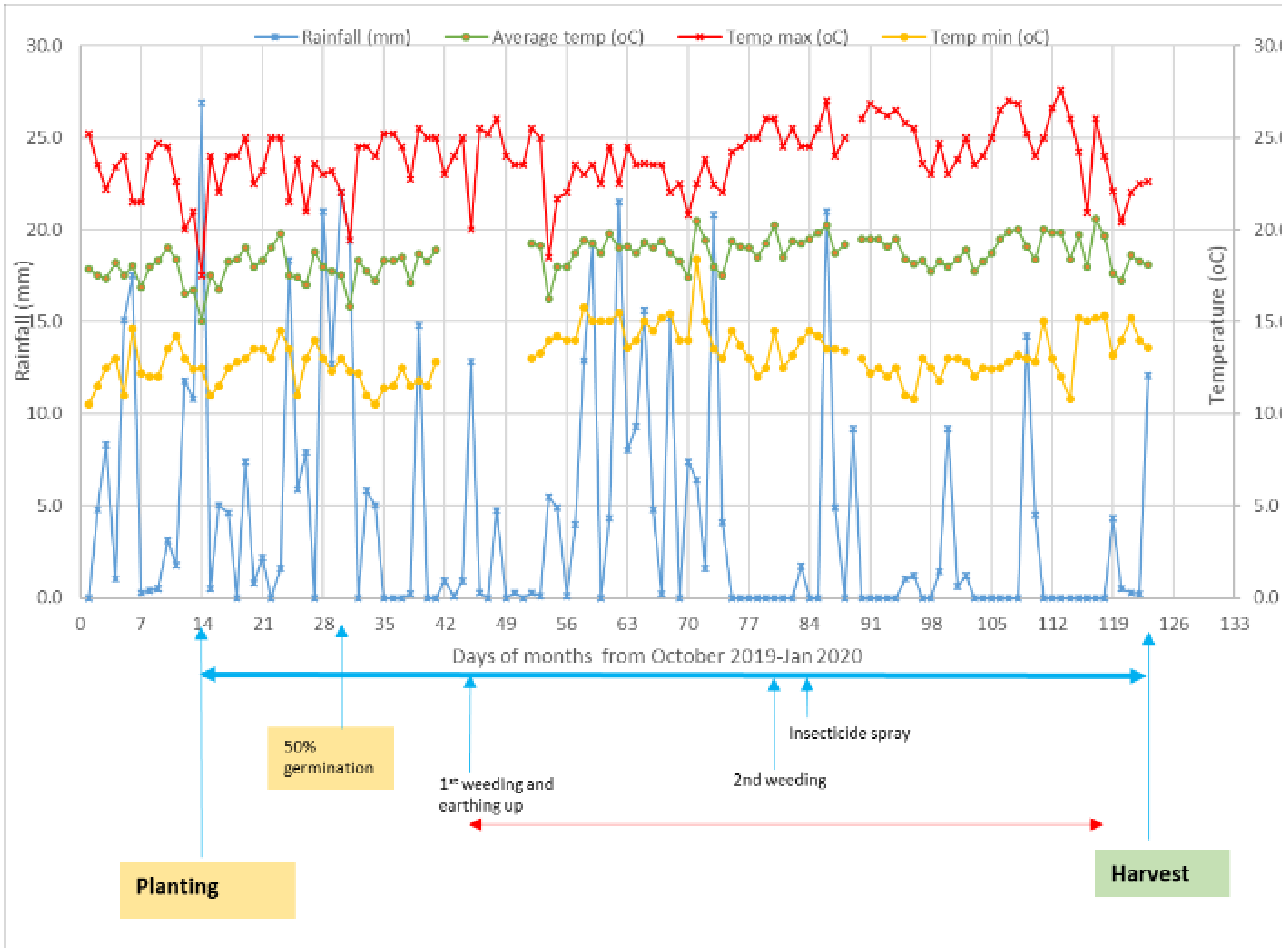

Figure S1a. Crop management of regulatory trials at Kachwekano ZARDI. Colored lines are as follows: rainfall (mm) in blue, maximum temperature (°C) in red, minimum temperature (°C) in yellow, and average temperature (°C) in green.

Table S1a. Weather data during the regulatory trials at Kachwekano ZARDI.

Kackwekano ZARDI					
Month	**Day**	**Rainfall (mm)**	**Temp max (ºC)**	**Temp min (ºC)**	**Average temp (ºC)**
October	1	0.0	25.2	10.5	17.9
	2	4.8	23.5	11.5	17.5
	3	8.3	22.2	12.5	17.4
	4	1.0	23.4	13.0	18.2

	5	15.1	24.0	11.0	17.5
	6	17.5	21.5	14.6	18.1
	7	0.3	21.5	12.2	16.9
	8	0.4	24.0	12.0	18.0
	9	0.5	24.7	12.0	18.4
	10	3.1	24.5	13.5	19.0
	11	1.8	22.6	14.2	18.4
	12	11.8	20.0	13.0	16.5
	13	10.8	21.0	12.4	16.7
	14	26.9	17.5	12.5	15.0
	15	0.5	24.0	11.0	17.5
	16	5.0	22.0	11.5	16.8
	17	4.6	24.0	12.5	18.3
	18	0.0	24.0	12.8	18.4
	19	7.4	25.0	13.0	19.0
	20	0.8	22.5	13.5	18.0
	21	2.2	23.2	13.5	18.4
	22	0.0	25.0	13.0	19.0
	23	1.6	25.0	14.5	19.8
	24	18.3	21.5	13.5	17.5
	25	5.9	23.8	11.0	17.4
	26	7.9	21.0	13.0	17.0
	27	0.0	23.6	14.0	18.8
	28	21.0	23.0	13.0	18.0
	29	12.7	23.2	12.3	17.8
	30	22.0	22.0	13.0	17.5
	31	19.4	19.4	12.3	15.9
November	32	0.0	24.5	12.2	18.4
	33	5.8	24.5	11.0	17.8
	34	5.0	24.0	10.5	17.3
	35	0.0	25.2	11.4	18.3
	36	0.0	25.2	11.5	18.4
	37	0.0	24.5	12.5	18.5
	38	0.2	22.7	11.5	17.1
	39	14.8	25.5	11.8	18.7
	40	0.0	25.0	11.5	18.3
	41	0.0	25.0	12.8	18.9
	42	0.9	23.0		
	43	0.1	24.0		
	44	0.9	25.0		
	45	12.8	20.0		
	46	0.3	25.5		
	47	0.0	25.2		

	48	4.7	26.0		
	49	0.0	24.0		
	50	0.3	23.5		
	51	0.0	23.5		
	52	0.3	25.5	13.0	19.3
	53	0.1	25.0	13.3	19.2
	54	5.5	18.5	14.0	16.3
	55	4.9	21.7	14.2	18.0
	56	0.1	22.0	14.0	18.0
	57	4.0	23.5	14.0	18.8
	58	12.9	23.0	15.8	19.4
	59	19.2	23.5	15.0	19.3
	60	0.0	22.5	15.0	18.8
	61	4.3	24.5	15.0	19.8
December	62	21.5	22.5	15.5	19.0
	63	8.0	24.5	13.6	19.1
	64	9.3	23.5	14.0	18.8
	65	15.6	23.6	15.0	19.3
	66	4.8	23.5	14.5	19.0
	67	0.2	23.5	15.2	19.4
	68	15.2	22.0	15.4	18.7
	69	0.0	22.5	14.0	18.3
	70	7.4	20.8	14.0	17.4
	71	6.4	22.5	18.4	20.5
	72	1.6	23.8	15.0	19.4
	73	20.8	22.4	13.5	18.0
	74	4.1	22.0	13.0	17.5
	75	0.0	24.2	14.5	19.4
	76	0.0	24.5	13.7	19.1
	77	0.0	25.0	13.0	19.0
	78	0.0	25.0	12.0	18.5
	79	0.0	26.0	12.5	19.3
	80	0.0	26.0	14.5	20.3
	81	0.0	24.5	12.5	18.5
	82	0.0	25.5	13.2	19.4
	83	1.7	24.5	14.0	19.3
	84	0.0	24.5	14.5	19.5
	85	0.0	25.5	14.2	19.9
	86	21.0	27.0	13.5	20.3
	87	4.9	24.0	13.5	18.8
	88	0.0	25.0	13.4	19.2
	89	9.2			
	90	0.0	26.0	13.0	19.5

	91	0.0	26.8	12.2	19.5
	92	0.0	26.5	12.5	19.5
January	93	0.0	26.2	12.0	19.1
	94	0.0	26.5	12.5	19.5
	95	1.0	25.8	11.0	18.4
	96	1.2	25.5	10.8	18.2
	97	0.0	23.6	13.0	18.3
	98	0.0	23.0	12.5	17.8
	99	1.4	24.7	11.8	18.3
	100	9.2	23.0	13.0	18.0
	101	0.6	23.8	13.0	18.4
	102	1.2	25.0	12.8	18.9
	103	0.0	23.5	12.0	17.8
	104	0.0	24.0	12.5	18.3
	105	0.0	25.0	12.4	18.7
	106	0.0	26.5	12.5	19.5
	107	0.0	27.0	12.8	19.9
	108	0.0	26.8	13.2	20.0
	109	14.2	25.2	13.0	19.1
	110	4.5	24.0	12.8	18.4
	111	0.0	25.0	15.0	20.0
	112	0.0	26.6	13.0	19.8
	113	0.0	27.6	12.0	19.8
	114	0.0	26.0	10.8	18.4
	115	0.0	24.2	15.2	19.7
	116	0.0	20.9	15.0	18.0
	117	0.0	26.0	15.2	20.6
	118	0.0	24.0	15.3	19.7
	119	4.3	22.1	13.2	17.7
	120	0.5	20.4	14.0	17.2
	121	0.3	22.0	15.2	18.6
	122	0.2	22.5	14.0	18.3
	123	12.1	22.6	13.6	18.1

Figure S1b. Crop management of regulatory trials at Rwebitaba ZARDI. Colored lines are as follows: rainfall (mm) in blue, maximum temperature (°C) in red, minimum temperature (°C) in yellow, and average temperature (°C) in green.

Table S1b. Weather data during the regulatory trials at Rwebitaba ZARDI.

Rwebitaba ZARDI					
Month	**Day**	**Rainfall (mm)**	**Temp max (°C)**	**Temp min (°C)**	**Average temp (°C)**
Oct	1	5.0	25.0	12.8	18.9
	2	0.0	24.0	13.4	18.7
	3	4.2	25.2	14.0	19.6
	4	8.0	23.6	16.0	19.8
	5	5.8	24.8	14.4	19.6
	6	3.0	25.0	16.0	20.5
	7	1.5	26.0	13.0	19.5
	8	0.5	27.0	14.6	20.8

	9	4.0	26.0	14.0	20.0
	10	12.0	24.0	15.0	19.5
	11	19.5	24.0	15.0	19.5
	12	1.6	21.2	16.0	18.6
	13	56.0	26.0	15.4	20.7
	14	2.5	22.0	15.4	18.7
	15	5.2	26.4	14.0	20.2
	16	7.2	25.6	15.0	20.3
	17	1.8	27.0	15.2	21.1
	18	20.0	24.0	14.2	19.1
	19	8.6	26.0	15.2	20.6
	20	13.5	25.0	14.2	19.6
	21	32.0	25.0	14.0	19.5
	22	0.0	25.0	13.0	19.0
	23	0.8	24.6	16.0	20.3
	24	0.0	25.0	16.0	20.5
	25	20.0	26.0	15.0	20.5
	26	28.0	24.0	16.0	20.0
	27	1.0	26.0	15.0	20.5
	28	2.0	24.2	16.0	20.1
	29	0.0	25.0	15.0	20.0
	30	36.5	24.0	16.0	20.0
	31	7.2	25.0	15.0	20.0
Nov	32	35.3	25.8	14.0	19.9
	33	0.0	25.6	14.0	19.8
	34	0.0	26.4	14.8	20.6
	35	0.0	26.0	14.8	20.4
	36	0.0	27.0	15.0	21.0
	37	38.2	25.4	17.4	21.4
	38	40.2	24.2	16.4	20.3
	39	35.0	25.0	14.8	19.9
	40	9.8	25.0	14.0	19.5
	41	0.0	26.4	15.0	20.7
	42	0.6	22.0	15.0	18.5
	43	5.0	24.0	14.8	19.4
	44	1.0	25.4	15.0	20.2
	45	11.6	24.8	16.0	20.4
	46	0.0	27.2	15.8	21.5
	47	1.1	26.0	15.8	20.9
	48	0.0	26.2	14.2	20.2
	49	2.0	27.0	16.0	21.5
	50	2.6	25.0	14.6	19.8
	51	6.5	23.0	14.0	18.5

	52	0.0	27.0	15.0	21.0
	53	6.0	22.0	15.0	18.5
	54	7.5	23.0	14.0	18.5
	55	0.0	24.0	15.0	19.5
	56	9.0	24.2	15.0	19.6
	57	0.0	25.2	14.8	20.0
	58	0.0	25.4	16.8	21.1
	59	7.0	25.0	16.0	20.5
	60	0.7	26.0	15.0	20.5
	61	8.0	25.0	15.0	20.0
Dec	62	14.0	25.4	14.0	19.7
	63	0.5	24.4	16.0	20.2
	64	2.0	24.0	16.0	20.0
	65	0.0	25.0	15.0	20.0
	66	0.0	26.0	16.6	21.3
	67	0.4	25.4	15.0	20.2
	68	0.2	23.0	15.0	19.0
	69	2.5	25.4	14.4	19.9
	70	0.0	25.0	15.0	20.0
	71	5.0	24.0	15.2	19.6
	72	12.4	24.0	16.0	20.0
	73	0.0	25.0	14.0	19.5
	74	6.7	26.0	14.0	20.0
	75	30.0	23.4	14.2	18.8
	76	4.0	24.0	14.0	19.0
	77	5.0	21.2	15.0	18.1
	78	45.7	25.2	14.4	19.8
	79	10.7	24.4	14.6	19.5
	80	0.0	26.0	15.8	20.9
	81	0.0	25.2	14.4	19.8
	82	0.0	26.0	16.0	21.0
	83	0.0	25.4	15.4	20.4
	84	0.0	24.4	15.4	19.9
	85	5.4	25.6	16.0	20.8
	86	1.2	25.8	16.4	21.1
	87	0.8	24.6	15.8	20.2
	88	0.0	26.0	14.6	20.3
	89	0.0	25.8	15.8	20.8
	90	0.0	25.6	14.0	19.8
	91	0.0	26.6	14.0	20.3
	92	0.0	26.0	15.4	20.7
Jan	93	0.0	26.4	14.0	20.2
	94	0.0	26.8	15.0	20.9

	95	0.0	26.4	16.2	21.3
	96	1.9	24.6	16.0	20.3
	97	17.5	22.2	16.4	19.3
	98	0.0	25.2	14.8	20.0
	99	0.0	25.2	14.8	20.0
	100	1.5	26.8	15.4	21.1
	101	0.6	24.8	16.6	20.7
	102	0.0	25.6	14.4	20.0
	103	0.0	26.0	17.6	21.8
	104	0.0	23.8	12.6	18.2
	105	4.7	24.6	15.0	19.8
	106	0.0	25.8	13.8	19.8
	107	0.0	26.8	14.0	20.4
	108	0.0	26.8	15.0	20.9
	109	0.0	26.6	16.2	21.4
	110	0.0	26.4	16.8	21.6
	111	0.0	25.0	14.8	19.9
	112	0.0	26.6	14.4	20.5
	113	0.0	26.6	17.2	21.9
	114	0.0	26.0	16.0	21.0
	115	0.0	26.0	16.0	21.0
	116	0.0	27.2	14.4	20.8
	117	0.0	27.0	15.8	21.4
	118	0.0	26.0	18.6	22.3
	119	0.0	27.2	16.8	22.0
	120	0.8	22.2	16.8	19.5
	121	4.8	25.0	16.4	20.7
	122	24.8	26.2	16.8	21.5
	123	9.6	22.0	16.8	19.4

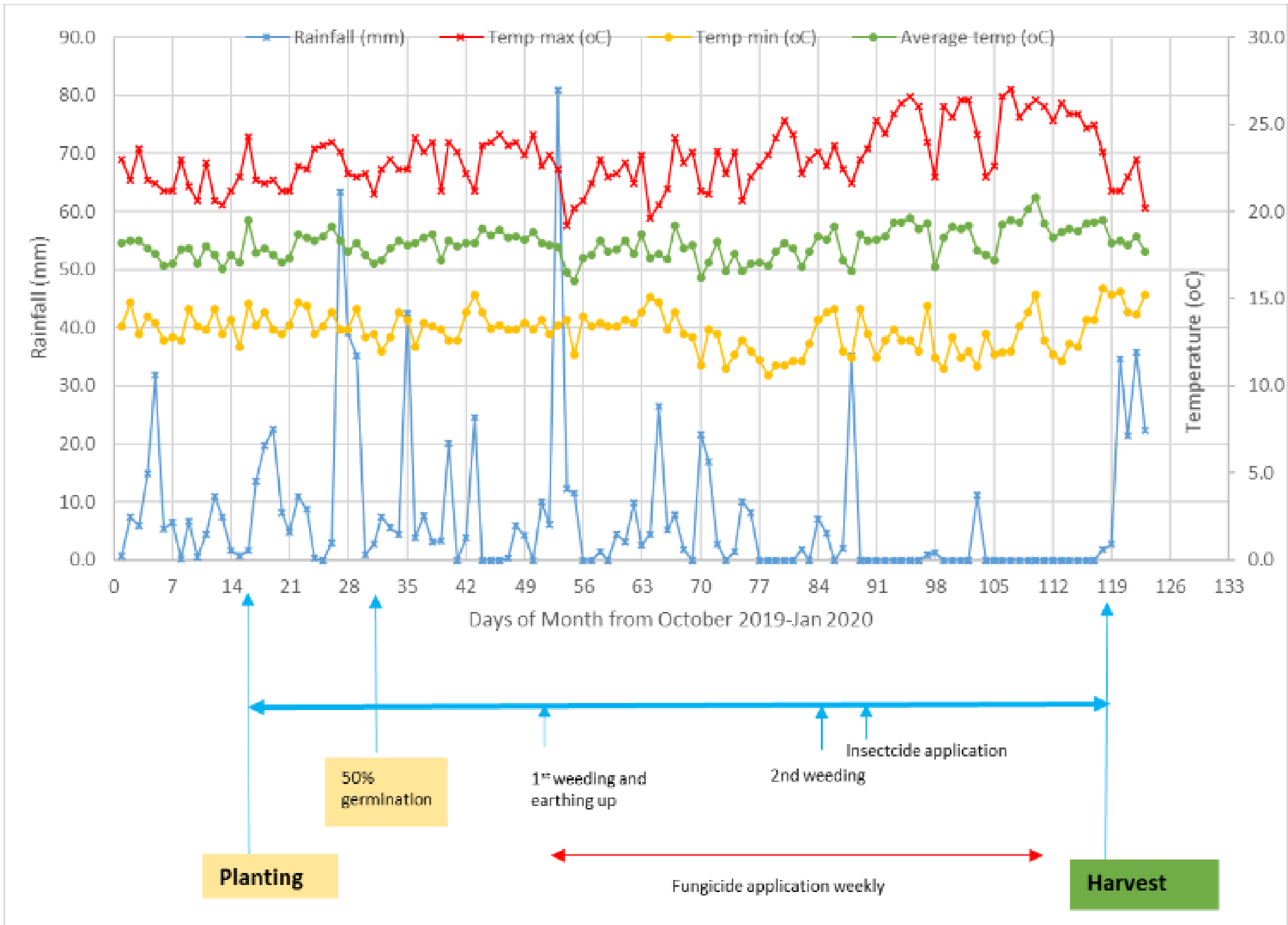

Figure S1c. Crop management of regulatory trials at Buginyanya ZARDI. Colored lines are as follows: rainfall (mm) in blue, maximum temperature (°C) in red, minimum temperature (°C) in yellow, and average temperature (°C) in green.

Table S1c. Weather data during the regulatory trials at Buginyanya ZARDI.

Buginyanya					
Month	**Day**	**Rainfall (mm)**	**Temp max (°C)**	**Temp min (°C)**	**Average temp (°C)**
Oct	1	0.7	23.0	13.4	18.2
	2	7.5	21.8	14.8	18.3
	3	5.9	23.6	13.0	18.3
	4	14.8	21.8	14.0	17.9
	5	31.9	21.6	13.6	17.6
	6	5.3	21.2	12.6	16.9
	7	6.4	21.2	12.8	17.0
	8	0.1	23.0	12.6	17.8
	9	6.6	21.4	14.4	17.9
	10	0.6	20.6	13.4	17.0

	11	4.4	22.8	13.2	18.0
	12	11.0	20.6	14.4	17.5
	13	7.5	20.4	13.0	16.7
	14	1.6	21.2	13.8	17.5
	15	0.7	22.0	12.2	17.1
	16	1.6	24.3	14.7	19.5
	17	13.5	21.8	13.5	17.7
	18	19.8	21.6	14.2	17.9
	19	22.5	21.8	13.2	17.5
	20	8.2	21.2	13.0	17.1
	21	4.8	21.2	13.5	17.4
	22	10.9	22.6	14.8	18.7
	23	8.8	22.4	14.6	18.5
	24	0.3	23.6	13.0	18.3
	25	0.0	23.8	13.4	18.6
	26	2.9	24.0	14.2	19.1
	27	63.3	23.4	13.2	18.3
	28	39.1	22.2	13.2	17.7
	29	35.2	22.0	14.4	18.2
	30	0.8	22.2	12.8	17.5
	31	2.7	21.0	13.0	17.0
Nov	32	7.5	22.4	12.0	17.2
	33	5.5	23.0	12.8	17.9
	34	4.5	22.4	14.2	18.3
	35	42.4	22.4	13.8	18.1
	36	3.8	24.2	12.2	18.2
	37	7.6	23.4	13.6	18.5
	38	3.2	24.0	13.4	18.7
	39	3.3	21.2	13.2	17.2
	40	20.1	24.0	12.6	18.3
	41	0.0	23.4	12.6	18.0
	42	3.8	22.2	14.2	18.2
	43	24.5	21.2	15.2	18.2
	44	0.0	23.8	14.2	19.0
	45	0.0	24.0	13.3	18.7
	46	0.0	24.4	13.5	19.0
	47	0.3	23.8	13.2	18.5
	48	6.0	24.0	13.2	18.6
	49	4.2	23.2	13.6	18.4
	50	0.0	24.4	13.2	18.8
	51	10.0	22.6	13.8	18.2
	52	6.2	23.2	13.0	18.1
	53	80.9	22.4	13.5	18.0

	54	12.3	19.2	13.8	16.5
	55	11.5	20.2	11.8	16.0
	56	0.0	20.6	14.0	17.3
	57	0.0	21.6	13.4	17.5
	58	1.5	23.0	13.6	18.3
	59	0.0	22.0	13.4	17.7
	60	4.5	22.2	13.4	17.8
	61	3.2	22.8	13.8	18.3
Dec	62	9.8	21.6	13.6	17.6
	63	2.5	23.2	14.2	18.7
	64	4.5	19.6	15.1	17.4
	65	26.5	20.4	14.8	17.6
	66	5.1	21.3	13.2	17.3
	67	7.8	24.2	14.2	19.2
	68	1.8	22.8	13.0	17.9
	69	0.0	23.4	12.8	18.1
	70	21.5	21.2	11.2	16.2
	71	16.9	21.0	13.2	17.1
	72	2.8	23.5	13.0	18.3
	73	0.0	22.2	11.0	16.6
	74	1.4	23.4	11.8	17.6
	75	10.0	20.6	12.6	16.6
	76	8.2	22.0	12.0	17.0
	77	0.0	22.6	11.5	17.1
	78	0.0	23.2	10.6	16.9
	79	0.0	24.2	11.2	17.7
	80	0.0	25.2	11.2	18.2
	81	0.0	24.4	11.4	17.9
	82	1.8	22.2	11.4	16.8
	83	0.0	23.0	12.4	17.7
	84	7.0	23.4	13.8	18.6
	85	4.7	22.6	14.2	18.4
	86	0.0	23.8	14.4	19.1
	87	2.0	22.4	12.0	17.2
	88	35.2	21.6	11.6	16.6
	89	0.0	23.0	14.4	18.7
	90	0.0	23.6	13.0	18.3
	91	0.0	25.2	11.6	18.4
	92	0.0	24.5	12.6	18.6
Jan	93	0.0	25.6	13.2	19.4
	94	0.0	26.2	12.6	19.4
	95	0.0	26.6	12.6	19.6
	96	0.0	26.0	12.0	19.0

	97	0.8	24.0	14.6	19.3
	98	1.2	22.0	11.6	16.8
	99	0.0	26.0	11.0	18.5
	100	0.0	25.4	12.8	19.1
	101	0.0	26.4	11.6	19.0
	102	0.0	26.4	12.0	19.2
	103	11.1	24.4	11.1	17.8
	104	0.0	22.0	13.0	17.5
	105	0.0	22.6	11.8	17.2
	106	0.0	26.6	11.9	19.3
	107	0.0	27.0	12.0	19.5
	108	0.0	25.4	13.4	19.4
	109	0.0	26.0	14.2	20.1
	110	0.0	26.4	15.2	20.8
	111	0.0	26.0	12.6	19.3
	112	0.0	25.2	11.8	18.5
	113	0.0	26.2	11.4	18.8
	114	0.0	25.6	12.4	19.0
	115	0.0	25.6	12.2	18.9
	116	0.0	24.8	13.8	19.3
	117	0.0	25.0	13.8	19.4
	118	1.8	23.4	15.6	19.5
	119	2.8	21.2	15.2	18.2
	120	34.7	21.2	15.4	18.3
	121	21.4	22.0	14.2	18.1
	122	35.8	23.0	14.1	18.6
	123	22.4	20.2	15.2	17.7